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Original Research Paper

Pathology

EFFECT OF IRON DEFICIENCY ANEMIA ON HbA1c IN DIABETES PATIENTS – A PROSPECTIVE STUDY

Surabhi Mishra 3rd year PG Resident, Department of Pathology, GR Medical College Gwalior, Madhya Pradesh, India

Arti Gupta* M.D. (Pathology), Associate Professor, Department of Pathology, GR Medical College, Gwalior, Madhya Pradesh, India. *Corresponding Author

ABSTRACT

Introduction- The diabetic burden has increased by more fast speed in low- and middle-income countries compared to high-income countries. HbA1c is affected by multiple factors like genetic factors, haematological factors and illness related factors. Initial studies suggested a relationship between HbA1c levels and iron deficiency anemia.. Our aim is to study the levels of HbA1c in iron deficiency anemia patients and the changes in HbA1c level after the correction of iron. **Material and methods-**2 ml sample of blood is collected from the consenting patient in an EDTA vial and plain vial each. The some fraction of the blood from EDTA vial is used for analysing complete blood count and hemoglobin the remaining sample from the EDTA vial is used for calculating the HBA1c value. The sample collected in the plain vial is used for testing the ferritin. **Results-** Significant negative low correlation was found between HbA1c and Ferritin; this means with increase in the values of HbA1c slight decrease in the value of Ferritin was observed and vice versa in this study. Similarly significant negative low correlation was found between Ferritin and Mean glucose, this means with increase in the values of Ferritin slight decrease in the value of Mean glucose was observed and vice versa in the study. Whereas, significant, strong positive correlation was found between HbA1c and Mean glucose, suggesting that with increase in the value of Mean glucose the values of HbA1c also increases and vice versa. **Conclusion-** While treating Diabetes Mellitus we have to kept in mind the haemoglobin status of patient. Correction of Iron Deficiency Anemia is important before treating Diabetes Mellitus to prevent misdiagnosis.

KEYWORDS :

INTRODUCTION:

Diabetes Mellitus (DM) is a heterogenic group of disorder characterized by hyperglycaemia because of either deficient insulin secretion, insulin action, or combination of both. Prolonged hyperglycaemia is associated with long term complications in several organ systems. DM cases can be subdivided into type 1 and type 2, the first one characterized by complete lack of insulin secretion by beta cells of pancreas and the second with insulin resistance on target organs accompanied by progressive deficiency of insulin secretion [1, 2]. The diabetic burden has increased by more fast speed in low- and middle income countries compared to high-income countries [3]. An increase in number of cases of DM type 2 is being correlated with obesity. In 2011 Haemoglobin a1c(HbA1c) recommended as a diagnostic tool for DM by the World Health Organization (WHO), with a cut point of 6.5 % (≥6.5 %) for diagnosis, but with a value beneath this cut point does not exclude DM when other glucose tests are used[3]. The HbA1c concentration formed after a non-enzymatic process in which glucose attaches to haemoglobin (Hb). Nathan et al found, a strong correlation between Hba1c level in a group of diabetic and non -diabetic individuals at weeks 8 and 12 with the continuous glucosemonitoring, a time period which corresponds to the approximate life span of an erythrocyte [4]. In addition, a strong correlation has been found between HbA1c level and development of DM-related complications [5].

A decrease in HbA1c levels found to diminish the risk of DM-related complications in type 2 diabetic patients [6]. In addition to possessing diagnostic value, HbA1c is also used for assessment of glycaemic control and used in diabetic therapy together with other glycaemic control measurements [7]. The reliability on HbA1c has been questioned time to time, not only regarding the use in children and adolescents[8,9], but also regarding the influencing factors capable of giving spurious HbA1c results, some of them adapted by the WHO from Gallagher et al[10]are erythropoiesis, altered Hb, glycation, erythrocyte destruction and assays. In the Standards of Medical Care in Diabetes from 2014 the American Diabetes Association (ADA) acknowledges the questions on the use of HbA1c in diagnosing children and adolescents, but concludes that they continue recommending HbA1c for diagnosis in these age groups [8, 9].HbA1c is affected by multiple factors like genetic factors, haematological factors and illness related factors. Initial studies suggested a relationship between HbA1c levels and iron deficiency anaemia. They tried to explain that on the basis of structural modifications and alterations in HbA1c levels in old and new red blood cells. Few studies reported no differences in the HbA1c levels of anaemic patients compared to healthy controls. Few studies stated that higher HbA1c levels were seen in iron deficiency anaemia patients and it decreased significantly after treatment. The results of various studies on relationship between HbA1c and iron deficiency anaemia were conflicting. Only fewer studies have been conducted in Indian population on this topic. Our aim is to study the levels of HbA1c in iron deficiency anaemia patients and the changes in HbA1c level after the correction of iron.Evidence that systemic iron overload would contribute to abnormal glucose metabolism was first derived from the observation that the incidence of Diabetes is increased in Classic Hereditary Hemochromatosis (Genetic iron overload state)[11].Even in conditions where overt iron overload is unrelated to any predisposed condition there is a relationship between high iron intake and incidence of Diabetes [11].

AIMS AND OBJECTIVE:

Aim of the present study is to evaluate Serum Ferritin relationship with HbA1c% and Blood glucose in patients in Type 2 Diabetes Mellitus. Our objectives were 1. To study the correlation between Serum Ferritin and Glycemic status by mean blood sugar level and HbA1c%. 2. To compare Serum ferritin among healthy controls of different age groups and Type 2 Diabetes Mellitus. 3. To study the effect of gender on Serum Ferritin. 4. To study whether increased HbA1C level is due to iron deficiency anemia or diabetes. 5. To prevent the misdiagnosis of diabetes with coexisting iron deficiency anemia.

MATERIAL AND METHODS:

This study was conducted in the department of Pathology G.R. Medical College, J.A. group of hospitals, Gwalior (MP). After taking approval for the study protocol from the Scientific

Review committee and the Ethical Review Committees to which G.R. Medical College is affiliated. A total of 100 study participants were included in the study. All the patients referred to the department of Pathology by the department of Medicine, were explained about the study in their vernacular language those who met the inclusion criteria and were consenting for additional 2ml blood sample collection for the ferritin examination were included in the study. It was a cross sectional observational study done for about one and a half year between January 2021 to May 2022. Sample size of the study was 100.

Inclusion Criteria:

1. All iron deficiency anemia patients, coming from medicine OPD of GRMC Gwalior (MP)
2. All the patient with diabetes mellitus, coming from medicine OPD of GRMC Gwalior (MP)

Exclusion Criteria:

1. Non diabetic patient.
2. Non anemic patient.
3. Uncooperative patient.
4. Patients not consenting for additional 2ml blood sample collection for the ferritin examination.

Descriptive analysis was done in the form of mean and standard deviations or proportions wherever appropriate. Statistical difference between means was calculated using the independent t test. Chi-square was used to analyze the difference between proportions. P value of less than 0.05 was considered statistically significant.

2 ml sample of blood was collected from the consenting patient in an EDTA vial and Plain Vial each. The some fraction of the blood from EDTA vial was used in the auto analyser machine **(D-5200 of Meditech)** for analysing Complete Blood Count and Hemoglobin The remaining sample from the EDTA vial was used in the Automated cation exchange HPLC **(ADAMS A1C of Arkray)** machine for calculating the HBA1c value. The Automated cation exchange HPLC machine has two racks the sample was placed in the anemic rack if the value of hemoglobin was <10 gm/dl, otherwise the sample was placed in the normal rack. The sample collected in the plain vial was allowed to coagulate the blood and then serum was separated and used for testing the ferritin levels in **(MAGLUMI Snibe 800 "CLIA")** which is a semiautomated machine.

OBSERVATIONS AND RESULTS:

Gender wise distribution of study participants

Gender	Frequency	Percentage
Male	37	37.0
Female	63	63.0
Total	**100**	**100.0**

Age wise distribution of study participants

Age Groups (Yrs)	Frequency	Percentage
10 - 19	3	3.0
20 - 29	17	17.0
30 - 39	9	9.0
40 - 49	20	20.0
50 - 59	**25**	**25.0**
60 - 69	22	22.0
70+	4	4.0
Total	**100**	**100.0**

Table showing hemoglobin values of the study participants

Hemoglobin(gm/dl)	Frequency	Percentage
<6.9	2	2.0
7 - <7.9	9	9.0
8 -8.9	12	12.0
9 -9.9	24	24.0
10 -10.9	**25**	**25.0**
11 -11.9	14	14.0
12 -12.9	9	9.0
>=13	5	5.0
Total	**100**	**100.0**

Table showing HbA1c values of the study participants

HbA1c (%)	Frequency	Percentage
<5.6	24	24.0
5.7- 6.4	19	19.0
6.5 - 8	18	18.0
>8	**39**	**39.0**
Total	**100**	**100.0**

Table showing Mean Glucose values of the study participants

Mean Glucose (mg/dl)	Frequency	Percentage
70 - 124.9	32	32.0
125 – 199.9	31	31.0
>200	**37**	**37.0**
Total	**100**	**100.0**

Table showing Diabetic status of the study participants

Diabetes Status	Frequency	Percentage
Diabetes Mellitus	84	84.0
Gestational Diabetes Mellitus	1	1.0
Non Diabetic	15	15.0
Total	**100**	**100.0**

Table shows the correlation between hemoglobin, HbA1c, Ferritin and Mean glucose value.

Correlation		Hb	HbA1c	Ferritin	Mean glucose
Hb	r (Pearson Correlation)	1			
	p value				
HbA_{1c}	r (Pearson Correlation)	-.050			
	p value	.622			
Ferritin	r (Pearson Correlation)	-.012	-.302**		
	p value	.909	<0.05		
Mean Glucose	r (Pearson Correlation)	-.052	.998**	-.302**	1
	p value	.611	<0.01	<0.05	

The above table shows that significant negative low correlation was found between HbA1c and Ferritin; this means with increase in the values of HbA1c slight decrease in the value of Ferritin was observed and vice versa in this study.

Similarly significant negative low correlation was found between Ferritin and Mean glucose, this means with increase in the values of Ferritin slight decrease in the value of Mean glucose was observed and vice versa in the study. Whereas, significant, strong positive correlation was found between HbA1c and Mean glucose, suggesting that with increase in the value of Mean glucose the values of HbA1c also increases and vice versa.

Table shows the correlation between Hemoglobin, HbA1c, Ferritin and Mean glucose value in Diabetic and Non-Diabetic participants

	Correlation		Hb	HbA1c	Ferritin	Mean glucose
Diabetic (n=85)	Hb	r (Pearson Correlation)	1			
		p value				
	HbA1c	r (Pearson Correlation)	-.085	1		
		p value	.442			
	Ferritin	r (Pearson Correlation)	-.005	-.384**	1	
		p value	.965	<0.01		
	Mean Glucose	r (Pearson Correlation)	-.087	.997**	-.385**	1
		p value	.430	<0.01	<0.01	
Non-Diabetic (n=15)	Hb	r (Pearson Correlation)	1			
		p value				
	HbA1c	r (Pearson Correlation)	-.034	1		
		p value	.904			
	Ferritin	r (Pearson Correlation)	-.016	-.070	1	
		p value	.954	.804		
	Mean Glucose	r (Pearson Correlation)	-.034	1.000**	-.070	1
		p value	.904	<0.01	.804	

DISCUSSION:

In our study majority of the study participants were females 63%. Whereas the age wise distribution of study participants in our study was, 25% (25/100) of the study participants belonged to 50-59 years. Mean age being 47.3± 15.7 years.

Momeni et al in her study conducted in **2015** found 67.2% patients were women, the mean age of the patients being 56.5 ± 9.7 (30 to 82) years. These findings are similar to the findings of our study [12]. In our study according to their hemoglobin value we found that majority (25%) of the study participants had hemoglobin value within range of 10 -10.9gm/dl, mean haemoglobin value being 10.11± 1.7 gm/dl. We did not observe any association between hemoglobin with HbA1c, ferritin or mean glucose values.

Brooks et al carried out a study in individuals with iron deficiency anemia. They estimated HbA1c values before and after treating them with iron. They noted that the mean concentration of HbA1c was elevated in iron deficiency anemia patients and it decreased after treatment. There was a postulation that the quarternary structure of the hemoglobin may be altered in iron deficiency anemia resulting in a higher rate of glycosylation of the globin chain. [13]

HbA1c distribution of study participants in our study we found out that majority (39%) had HbA1c value more than 8%; Mean HbA1c value being 7.85± 2.7%. Most of the study participants have HbA1c value more than 6.5 that indicates poor diabetic control. We found out that amongst males (32 /37) 86.5% have ferritin levels between 15-300 mg/dl, only one male participant had ferritin level below 15 mg/dl whereas, amongst females (46 /63) 86.5% have ferritin levels between 15-200 mg/dl, (10/63) 11.1% female participants had ferritin level below 15 mg/dl. **Forouhi et al.** in **2007** stated that there was no evidence of a statistical interaction between ferritin levels and sex (p = 0.51), and analyses were performed in men and women combined.[14] In our study significant negative low correlation was found between HbA1c and Ferritin; this means with increase in the values of HbA1c slight decrease in the value of Ferritin was observed and vice, significant negative low correlation was also found between HbA1c and Ferritin among diabetic participants; and among male participants of the study(significant negative moderate correlation). **Pettersen** in a cross section design in **2018** found, the relation between iron deficiency and anemia and HbA1c. He found significant higher HbA1c levels in adolescents with anemia and iron deficiency, compared to adolescents without these conditions, respectively. Some gender differences in the subgroups of anemia were also found. No association was found with fasting glucose levels and HOMA-IR in iron deficient- or anemic adolescents. They stated that HbA1c must be assessed with caution in adolescents, when anemia or iron deficiency is present. [15] **Momeni et al in 2015** stated that there was a significant negative correlation between serum ferritin and duration of diabetes (r = 0.259; P = 0.034). [12]

In 2010, Koga et al. carried out a study in premenopausal women to find out the relation between the indices of iron metabolism and HbA1c. According to this study there was an inverse association between the H bA1c and the serum iron, serum transferrin saturation and serum ferritin. Higher HbA1c levels were observed in iron deficiency anemia group than in the normal iron state group. This study concluded that in 53 premenopausal women the regardless of anemia, iron deficiency increases HbA1c levels. [16]

We observed significant, strong positive correlation between HbA1c and Mean glucose, suggesting that with increase in the value of Mean glucose the values of HbA1c also increases and vice versa. Strong positive correlation was also found between HbA1c and Mean glucose in both the diabetic as well as non diabetic study participants.

Sluiter et al. postulated that the glycosylation of hemoglobin is an irreversible process and the concentration of HbA1c in a red blood cell increases with cell age. The levels of HbA1c should be normal in individuals with normal glycemic status and normal red blood cell life span. In the event of chronic iron deficiency anemia, red blood cell production will decrease leading to anemia and a longer span for the red blood cells present in the circulation. After treating with iron, HbA1c levels will decrease which is attributable to the phenomenon shorter life span of red blood cells. [17]

Significant negative low correlation was found between Ferritin and Mean glucose in our study, this means with increase in the values of Ferritin slight decrease in the value of Mean glucose was observed and vice versa. Similarly significant negative low correlation was also found between Ferritin and Mean glucose among diabetic participants. **Forouhi et al in 2007** confirmed in their studies that poorly controlled diabetes patients had hyper-ferritinemia. This showed that serum ferritin was increased in diabetes as long as glycemic control was not achieved. [14]

CONCLUSION:

We concluded that the Prevalence of Iron Deficiency Anemia is more in females and low levels of ferretin is associated with low Haemoglobin. High HbA1C levels are present in patient of uncontrolled Diabetes Mellitus. In this study we found that

with increase in the values of HbA1c there is slight decrease in the value of Ferritin and vice versa. So while treating Diabetes Mellitus we have to keep in mind the haemoglobin status of patient. Correction of Iron Deficiency Anemia is important before treating Diabetes Mellitus to prevent misdiagnosis. However large sample size and longer duration of time is needed to evaluate the correlation more precisely.

REFERENCES:

1. Association AD. Diagnosis and classification of diabetes mellitus. Diabetes care. 2014;37 Suppl1:S81-90.
2. Association AD. Classification and Diagnosis of Diabetes. In: 2016 Standards of Medical Care in Diabetes. Diabetes Care 2016 [cited 2016 11 October]. Available from: http://care.diabetesjournals.org/ content/ diacare/39/ Supplement_1/S13.full.pdf.
3. Organization. WH. WHO Guidelines Approved by the Guidelines Review Committee. Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus: Abbreviated Report of a WHO Consultation. Geneva: World Health Organization Copyright (c) World Health Organization 2011; 2011.
4. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia. 2007;50(11):2239-44
5. Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R, et al. Medical management of hyperglycaemia in type 2 diabetes mellitus: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement from the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia. 2009;52(1):17-30.
6. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35):prospective observational study. BMJ (Clinical research ed). 2000;321(7258):405-12.
7. Chamberlain JJ, Rhinehart AS, Shaefer CF, Jr., Neuman A. Diagnosis and Management of Diabetes: Synopsis of the 2016 American Diabetes Association Standards of Medical Care in Diabetes. Annals of internal medicine. 2016;164(8):542-52.
8. Association AD. Standards of medical care in diabetes--2014. Diabetes care. 2014;37 Suppl1:S14-80.
9. Kester LM, Hey H, Hannon TS. Using hemoglobin A1c for prediabetes and diabetes diagnosis in adolescents:can adult recommendations be upheld for pediatric use? The Journal of adolescent health: official publication of the Society for Adolescent Medicine. 2012;50(4):321-3.
10. Gallagher EJ, Le Roith D, Bloomgarden Z. Review of haemoglobin A(1c) in the management of diabetes. Journal of diabetes. 2009;1(1):9-17.
11. Jehn M, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in US adults. Diabetes care. 2004 Oct 1;27(10):2422-8.
12. Momeni A, Behradmanesh MS, Kheiri S, Abasi F. Serum ferritin has correlation with HbA1c in type 2 diabetic patients. Adv Biomed Res. 2015 Mar25;4:74.
13. Brooks AP, Metcalfe J, Day JL, Edwards MS. Iron deficiency and glycosylated haemoglobin A. Lancet 8186(2):141, 1980.
14. Forouhi NG, Harding AH, Allison M, Sandhu MS, Welch A, Luben R, et al. Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. Diabetologia. 2007 May 1;50(5):949–56.
15. Pettersen LD. The relation between iron deficiency and anaemia and HbA1c levels in Norwegian adolescents. The Tromsø Study: Fit Futures. 2018 Jun 4 [cited 2022 Sep 24]; Available from: https://munin.uit.no/handle/10037/18459
16. Hussain N. Implications of using HBA1C as a diagnostic marker for diabetes. Diabetol Int. 2016 Mar 1;7(1):18–24.
17. Rabani S, Sardarinia M, Akbarpour S, Azizi F, Khalili D, Hadaegh F. 12 year trends in cardiovascular risk factors (2002-2005 through2011-2014) in patients with cardiovascular diseases: Tehran lipid and glucose study. PLOS ONE. 2018 May 16; 13(5):e0195543.